1325-Pos Board B95

Structural Differences Between Postsynaptic Densities Isolated from Different Brain Regions

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The postsynaptic density (PSD) is a large protein complex that works to cluster neurotransmitter receptors at the synapse needed for synaptic transmission and to organize the intracellular signaling molecules responsible for altering the efficiency of synaptic transmission - termed synaptic plasticity. We propose that different synapses from different parts of the brain place unique demands on the process of synaptic transmission and that the structure and composition of the PSD play a role in providing these unique properties. To begin to address this question, PSDs were isolated from adult rat cerebella, hippocampi and cortices, three brain areas amenable to straightforward isolation that contain unique distributions of neuronal cell types. Cryo-tomography and immunogold labeling were utilized to quantify protein composition and distribution. Although the mean surface area did not significantly differ between PSD types, the variance was significantly larger for cerebellar PSDs. Labeling densities for PSD-95 and αCaMKII were found to differ dramatically among the PSD types, while all regions were found to have moderate to high labeling for βCaMKII, illustrating the importance of βCaMKII to the PSD structure. PSD-95, a common PSD marker and scaffold protein, was absent from a fraction of cerebellar PSDs, unlike hippocampal and cortical PSDs, showing that protein composition varies between PSD types. Qualitatively, PSD-95 was found to cluster in cerebellar PSDs unlike other PSD types, suggesting a different function for PSD-95 in cerebellar PSDs. Ripley's K function analysis is currently being implemented to evaluate gold particle distribution. These results support the idea that the composition and structure of the PSD change with brain region, possibly to achieve the specific synaptic functions required of each brain region.

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Rapid Assembly of a Multimeric Membrane Protein Pore Observed by Single Molecule Fluorescence

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Pore-forming proteins play a vital role in the eukaryotic immune response, where immune surveillance can lead to the targeted-attack of infected cells and bacteria via pore generation. Conversely, many bacteria use similar protein pores to kill target cells and acquire nutrients from their host. However, the mechanisms by which many of these multimeric membrane proteins assemble to form pores are not well understood. We have observed the assembly of the staphylococcal pore-forming toxin α-hemolysin using single-molecule fluorescence imaging. Surprisingly, assembly from the monomer to the complete heptamer is extremely rapid, occurring in less than 5 ms. No lower-order oligomeric intermediates are detected. Monte Carlo simulation of our experiment shows that assembly is diffusion-limited, and pore formation is dependent on the stability of intermediate species. There are close similarities between bacterial pore-forming toxins, such as staphylococcal α-hemolysin, the anthrax protective antigen and the cholesterol-dependent cytolysins (CDCs), and their eukaryotic analogues, such as the complement pore - membrane attack complex and perforin domain (MACPF). The assembly mechanism we have observed for α-hemolysin provides a simple model that aids our understanding of these important pore-formers.

To image rapidly diffusing α-hemolysin monomers and observe their assembly into higher-order oligomers requires bilayer longevity, high signal-to-noise, high time resolution and control of lipid composition. We have recently developed a new synthetic mimic of the cell membrane that fulfils these requirements; we form a Droplet Interface Bilayer by contact of two lipid monolayers between a nanolitre aqueous droplet and a hydrogel support immersed in a solution of phospholipid in hexadecane. To monitor the entire assembly process we introduced Cy3b-labeled α-hemolysin monomers into the droplet using a piezo-driven nanoinjector. The labeled α-hemolysin species were imaged on the bilayer using total internal reflection fluorescence (TIRF) microscopy.

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Responsive and Tunable Neurofilament Protein Hydrogel Assemblies - A Synchrotron X-Ray Scattering Study of Composition and Salt Dependent Response

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Neurofilaments (NFs) are the intermediate filaments in neuronal cells that along with other cytoskeletal network structures play a major role in the mechanical integrity of neuronal processes. NFs are assembled from three different subunits (NF-Low (NF-L), NF-Medium (NF-M), NF-High (NF-H)) that differ mainly in the sequence length of their unstructured C-terminal sidearms. The sidearms direct the lateral associations between filaments thus forming the NF hydrogel networks (the physiologically relevant NF protein assembly state in the axoplasm). The interfilament lateral associations are predominantly electrostatic, enabled by the polyampholytic nature of the sidearms. We examine their strength and range by varying the salinity of the in vitro buffer. Furthermore, motivated by variable in vivo subunit expression in axons versus dendrites that results in variable network packing, reassembled (in vitro) binary system NF-hydrogels have revealed the different contributions of individual subunits to interfilament interactions and to network interfilament spacings [1]. Synchrotron x-ray scattering experiments have allowed us to study the changes in the microscopic structure of NF hydrogels as a function of salt and sidearm density. At high weight ratios of NF-M and NF-H, and as a function of increasing salt concentrations, NF gels exhibit an unexpectedly abrupt transition from a weakly oriented (nearly isotropic) low filament density gel with interfilament spacing d=1000Å to a highly oriented liquid crystalline gel with high filament density and d=500Å (NF-M) and 700Å (NF-H). The tunability of the network in vitro mirrors in vivo cellular control of the NF network via subunit phosphorylation, which may transition the network from a highly oriented rigid state to one with orientational plasticity.

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1328-Pos Board B98

In Vitro Expression of E. Coli Flagellar Nano-Motor Proteins using Cell Free Expression: A Path through a Controlled Re-Assembly of the Flagellum Basal Body

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The bacterial flagellum is a marvel of Nature. Composed of thousands of proteins, it propels its host using only a set of 6 kinds of proteins, assembled into the basal body. In vivo and in vitro studies revealed some aspect of it, in term of mechanical efficiency; proteins structures and part of its mechanism were highlighted by large-scale mutagenesis. However, a precise description of the motor's mechanism remains to be established. We report the use of cell free expression of several motor proteins, expressed into a ''proteocell''. A cell free system has been encapsulated into a lipid bilayer, forming a vesicle reactor-cell. By tracking the localization of the proteins using a fluorescence tag, 6 basal body proteins FliF, FliG, FliM FliN MotA and MotB were localized. In order to validate our approach, interaction between FliF and FliG was studied and confirmed by the use of truncated mutants of FliG showing no affinity for FliF. These results represent a novel and ambitious approach for studying in vitro complex architecture machinery as the flagellum nanomotor.